Human inhalation exposure to ethylene glycol*

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* This publication is dedicated to Prof. Dr. Dr. Hermann M. Bolt on the occasion of his 60th birthday.

Abstract Two male volunteers (A and B) inhaled 1.43 mmol and 1.34 mmol, respectively, of vaporous ^{13}C-labeled ethylene glycol ($^{13}C_2$-EG), over 4 h. In plasma, $^{13}C_2$-EG and its metabolite $^{13}C_2$-glycolic acid ($^{13}C_2$-GA) were determined together with the natural burden from background GA using a gas chromatograph equipped with a mass selective detector. Maximum plasma concentrations of $^{13}C_2$-EG were 11.0 and 15.8 µmol/l, and of $^{13}C_2$-GA were 0.9 and 1.8 µmol/l, for volunteers A and B, respectively. Corresponding plasma half-lives were 2.1 and 2.6 h for $^{13}C_2$-EG, and were 2.9 and 2.6 h for $^{13}C_2$-GA. Background GA concentrations were 25.8 and 28.3 µmol/l plasma. Unlabeled background EG, GA and oxalic acid (OA) were detected in urine in which the corresponding ^{13}C-labeled compounds were quantified, too. Within 28 h after start of the exposures 6.4% and 9.3% $^{13}C_2$-EG, 0.70% and 0.92% $^{13}C_2$-GA, as well as 0.08% and 0.28% $^{13}C_2$-OA of the inhaled amounts of $^{13}C_2$-EG were excreted in urine by volunteers A and B, respectively. The amounts of $^{13}C_2$-GA represented 3.7% and 14.2% of background urinary GA excreted over 24 h (274 and 88 µmol). The amounts of $^{13}C_2$-OA were 0.5% and 2.1% of background urinary OA excreted over 24 h (215 and 177 µmol). From the findings obtained in plasma and urine and from a toxicokinetic analysis of these data, it is highly unlikely that EG workplace exposure according to the German exposure limit (MAK-value: 10 ppm EG, 8 h) could lead to adverse effects from the metabolically formed GA and OA.

Key words *Ethylene glycol, Glycolic acid, Oxalic acid, Human, Risk, Metabolism, Toxicokinetics*

Introduction

Ethylene glycol (EG) is a widely used liquid with a low vapor pressure of 0.08 mbar at 20°C (DFG 1991). It is employed for antifreeze formulations, deicing of airplanes, production of polyesters and a series of other applications.

Figure 1

High doses of EG, a simplified metabolic scheme of which is given in Figure 1, show various toxic effects. Symptoms related to the central nervous system, metabolic acidosis, nephrotoxic and embryotoxic properties are characteristic (Andrews and Snyder 1991). Nephrotoxicity is ascribed to acidic metabolites as glycolic acid (GA) and oxalic acid (OA) (reviewed in e.g. LaKind et al. 1999). Both acids showed also developmental toxicity in rat embryo cultures (Klug et al. 2001). In vivo, EG exposed rats produced only minor amounts of OA in comparison with GA (Frantz et al. 1996). Embryotoxicity in EG and GA treated rats was related to GA (Carney et al. 1999). In rats, receiving EG in their food over two years, No-Observed-Effect-Levels (NOEL) for EG-induced nephrotoxicity were at 0.2% in food (about 80 mg/kg bw/d/2y; Blood 1965) and at 0.5% (about 200 mg/kg bw/d/2y; DePass et al. 1986). For B6C3F1 mice, the corresponding NOEL was 0.6% in food (about 1500 mg/kg bw/d/2y) (NTP 1993, cited in LaKind et al. 1999). NOELs for developmental toxicity have been reported to be 500 mg EG/kg bw/d in CD rats and 150 mg/kg bw/d in CD-1 mice (administration by gavage from gestation day 6 to gestation day 15; both species, Neeper-Bradley et al. 1995). For rats, the NOEL dose of 80 mg/kg bw can be calculated to lead to a maximum GA concentration in blood of 144 µmol/l (linear extrapolation from the maximum GA blood concentration of 271 µmol/l reached after a dose of 150 mg

EG/kg bw; Pottenger et al. 2001). The dose of 500 mg EG/kg bw resulted in a maximum GA concentration of 1723 µmol/l blood (Pottenger et al. 2001).

In the National Occupational Exposure Survey, conducted during 1981-1983, it was estimated that about 1.5 million workers were potentially exposed to EG in the USA, each year (NIOSH 1990, cited in IPCS 2002). Exposure may lead to uptake of EG. Only limited experimental or occupational data are available on humans exposed to EG in spite of its occupational relevance. Urinary EG was found in volunteers exposed (20-22 h/d, 30 d) to weekly air concentrations of EG between 17 and 49 mg/m3 (Wills et al. 1974), in workers using EG for de-icing of airplanes (Gérin et al. 1997) and in motor servicing workers (Laitinen et al. 1995). None of these studies established a reliable correlation between EG concentrations in urine and air. In order to obtain such a relationship and to enable a comparison between background and additional exposure-derived burdens, we determined EG, GA and OA and their $^{13}C_2$-labeled analogues in plasma and urine of human volunteers inhaling small amounts of vaporous $^{13}C_2$-EG at exposure conditions below the German MAK-value (maximum workplace concentration; EG: 10 ppm = 25.7 mg/m^3; DFG 1991).

Materials and methods

Chemicals

$^{13}C_2$-EG (99 atom% 13C) was obtained from Aldrich (Steinheim, Germany). All other chemicals were of analytical grade.

Exposure experiments

The study was approved by the ethics committee of the Technical University, Munich. Two healthy, non-smoking, male volunteers (A: 54 years old, 96 kg, B: 44 years old, 57 kg) avoided oxalate and ascorbate rich foods for 2 days before the individual experiments. On the exposure days, each volunteer inhaled vaporized $^{13}C_2$-EG from an all-glass vessel (Figure 2). Vapors of $^{13}C_2$-EG were generated by repeatedly (16 times, intervals of 15 min, 4 h) injecting small amounts of liquid $^{13}C_2$-EG (6.6 µl each) into the warmed, closed vessel. At the beginning of the time intervals, the volunteer inhaled the generated vapor via the mouth. Residual $^{13}C_2$-EG was determined from the vessel at the end of exposure.

Figure 2

During exposure, exhaled air was collected in gasbags (polyethylene coated aluminum bags, Linde, Unterschleißheim, Germany) and analyzed for $^{13}C_2$-EG. Prior to the exposure, an i.v. indwelling cannula (B.Braun, Melsungen, Germany) was positioned in the cubital vein. Samples of about 5 ml venous blood were collected before start of the exposure, during the exposure at each time interval of 15 min (about 7 min following each inhalation process) and up to 4 h post-exposure. Blood pH was determined in each sample. Plasma, obtained by centrifugation, was stored at -80°C until analyzed for labeled and unlabeled EG and GA. All urinary fractions were collected immediately prior to start of exposure and up to 30 h thereafter. Urinary pH was monitored immediately in each fraction, which was acidified subsequently. Then, fractions were stored at +4°C up to 24 h. Within this period, specimens were taken for the determination of labeled and unlabeled OA. Immediately thereafter, fractions were stored at -80°C until analyzed for labeled and unlabeled EG and GA. A five-week

stability test demonstrated that the storage duration did not influence the extent of recovery of the analytes.

Analysis of EG, GA and OA by gas chromatography

A detailed description of the analytical procedures is in preparation and will be submitted elsewhere. Therefore, only a brief description is given.

A gas chromatograph equipped with a mass selective detector (GC/MSD) was used for all analyses (GC: HP5890 Series II with cool-on-column inlet equipped with a HP-5MS column, 30 m length, 0.25 mm i.d., 0.25 mm film, carrier gas Helium; MSD: HP5972, electron impact ionization, 70 eV, selective ion monitoring). The complete system was obtained from Agilent Technologies, Waldbronn, Germany.

Sample preparation for EG

Both, propylene glycol and 1,3-propandiol served as internal standards in parallel. EG and the standards in the samples were derivatized with n-butylboronic acid, extracted with ethyl acetate and analyzed (limits of quantification in plasma: EG 7.6 µmol/l, $^{13}C_2$-EG 0.6 µmol/l; in urine: EG 1.1 µmol/l, $^{13}C_2$-EG 1.6 µmol/l).

Sample preparation for GA

Deuterated succinic acid (D_6-SA) and 2-hydroxyisovaleric acid were added as internal standards to plasma and to urine, respectively. The corresponding samples were deproteinized with acetonitrile (only plasma) and dried using a vacuum concentrator. The residues were treated with *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide

(MTBSTFA) and the derivatives were analyzed (limits of quantification in plasma: GA 1.2 µmol/l, $^{13}C_2$-GA 0.8 µmol/l; in urine: GA 32.6 µmol/l, $^{13}C_2$-GA 2.9 µmol/l).

Sample preparation for OA

Succinic acid and D_6-SA served as internal standards for the determination of OA and of $^{13}C_2$-OA, respectively. After adding these standards, the samples were acidified and extracted with ethyl acetate. The residues, obtained after drying with a vacuum concentrator, were silylated with MTBSTFA and the derivatives were analyzed (limits of quantification in urine: OA 17.2 µmol/l, $^{13}C_2$-OA 0.2 µmol/l).

Results

The volunteers did not report any effects related to the exposure. The inhaled $^{13}C_2$-EG was completely taken up from the inhalation air as proven by its absence in the expired air. Therefore, the amount taken up during the entire exposure period equaled the difference between the sum of the administered doses and the residual $^{13}C_2$-EG, which was determined in the glass vessel at the end of exposure. The pH measurements in blood and urine showed normal physiological values being 7.45 ± 0.024 (A) and 7.39 ± 0.027 (B) and ranging from 6 to 7.5 (A) and from 6 to 8 (B), respectively. In plasma of the volunteers, $^{13}C_2$-EG, $^{13}C_2$-GA and GA could be determined. Background EG was not found (limit of quantification 7.6 µmol/l). This high value probably resulted from interference by other compounds in plasma. There was no interference for the determination of $^{13}C_2$-EG. In urine of both volunteers, labeled and unlabeled EG, GA and OA were quantifiable.

Table 1 shows the inhaled doses of $^{13}C_2$-EG, both total [mmol] and normalized for body weight of each volunteer [mg/kg bw].

Table 1

Figure 3 depicts the concentration-time courses of $^{13}C_2$-EG in plasma during and after exposure. The ratio of the maximum $^{13}C_2$-EG concentrations found in volunteer A to that in B reflects that of the doses as given in Table 1. The half-lives of $^{13}C_2$-EG, derived from the elimination phase of the Figure, are 2.1 h (A) and 2.6 h (B), respectively. Twenty-four hours after start of the exposure $^{13}C_2$-EG was no longer detectable.

Figure 3

Figure 4 displays the concentration-time courses of $^{13}C_2$-GA in plasma during and after exposure. The maximum $^{13}C_2$-GA concentrations were reached shortly after the end of exposure (A: about 1 h; B: 0.5 h). These short time spans are in agreement with the observation that the $^{13}C_2$-GA half-lives of 2.9 h (A) and 2.6 h (B) were similar (A) or identical (B) with those of $^{13}C_2$-EG. The maximum $^{13}C_2$-GA concentration was lower in volunteer A than in B as it was observed for the maximum $^{13}C_2$-EG concentration, too (see above). The background concentrations of plasma GA were 25.8 ± 3.7 µmol/l and 28.3 ± 2.8 µmol/l for volunteer A and B, respectively.

Figure 4

Figures 5, 6 and 7 depict the cumulative amounts of unlabeled (background) and labeled EG, GA and OA excreted in the urine collected during and after exposure.

Figure 5

Figure 6

Figure 7

Table 2 shows the amounts of $^{13}C_2$-EG, $^{13}C_2$-GA and $^{13}C_2$-OA in urine, expressed as a percentage of the inhaled dose of $^{13}C_2$-EG (see Table 1). Obviously, EG is predominantly biotransformed to other metabolites presumably via intermediary metabolism. This conclusion is supported by data from rat studies. Following a 30 min inhalation exposure of Fischer-344 rats to gaseous ^{14}C-EG with a mean air concentration of 12.5 ppm, Marshall and Cheng (1983) determined the amounts of exhaled $^{14}CO_2$. From the initial ^{14}C-burden, about 30% was exhaled as $^{14}CO_2$ within the first 12 h. This metabolite amounted to 60% after 4 days.

Table 2

Table 3 gives the amounts of background GA and OA excreted over 24 h. It shows also the relative increases in these acids as labeled metabolites formed from inhaled $^{13}C_2$-EG the dose of which was 1.43 mmol (A) and 1.34 mmol (B).

Table 3

Discussion

Background values

EG: Background EG was quantifiable in urine (A: 18.2 µmol/24 h; B: 24.5 µmol/24 h). These urinary EG excretions can be compared with ratios of urinary EG to urinary creatinine reported by other groups for non-exposed persons (Laitinen et al. 1995;

Gérin et al 1997; Laitinen et al. 1997; Letzel et al. 2000). Using these ratios and considering a normal creatinine excretion in healthy men of 1.8 g/24 h (Geigy 1975), EG excretions can be calculated to be between 0 and 77 µmol/24 h. These amounts match with those measured in volunteers A and B. In the study of Wills et al. (1974) EG background concentrations have been reported for both serum (1500 – 3400 µmol/l) and urine (260 – 1200 µmol/l). The values seem to be very high for unexposed controls, in the light of the other published data on urinary EG. The difference might result from the rather unspecific methodology used by Wills et al. (1974), which was based on the periodate oxidation of EG to formaldehyde used by Russell et al. (1969). Since treatment with this reagent transforms a number of substances present in biological material into formaldehyde (Russell et al. 1969) an overestimation of EG probably occurred. Background EG could result from several sources. Endogenous and environmental ethylene and ethylene oxide could contribute via metabolism to background EG (reviewed in IARC 1994; Filser et al. 1994). A relevant EG source could be ethylene and ethylene oxide uptake via smoking (e.g. Törnqvist et al. 1986; Filser et al. 1992). A series of further possible sources for background EG, including food and consumer products, are discussed in IPCS (2002).

GA: The GA background concentrations in plasma determined in the present study are in agreement with those (6.6 - 32.9 µmol/l) published by Chalmers et al. (1984). Lower GA background concentrations (between 4.4 and 12.2 µmol/l) were reported by other authors in plasma of fasted (Maeda-Nakai and Ichiyama 2000) and in plasma-ultrafiltrate of fasted (Hagen et al. 1993) and non-fasted individuals (Petrarulo et al. 1991). Urinary background amounts of GA, excreted over 24 h (Table 3) are consistent with values from 75 to 1220 µmol/24 h published by a series of other

groups for healthy humans (Petrarulo et al. 1998 (review); Niederwieser et al. 1978; Marangella et al. 1992; Holmes et al. 1993; Maeda-Nakai and Ichiyama 2000). Only one publication reported a wider range from 0 to 1400 µmol/24 h (Hagen et al. 1993). Background GA can originate from several sources. GA is formed in the catabolism of proteins (Holmes et al. 1993) and carbohydrates (McWinney et al. 1987). It can also be taken up directly via food since it has been detected as natural constituent of vegetables, fruits and meat (Harris and Richardson 1980). Furthermore, GA is an ingredient of certain cosmetic products (reviewed in NICNAS 2000). A daily intake of about 1 mg GA/kg bw via food was estimated (DuPont 2002). Probably, the much higher GA amounts excreted in urine result from intermediary metabolism.

OA: Unfortunately, we were not able to quantify OA in plasma because the recovery in plasma was not reproducible. The reason for this behavior is unclear. Recently, several methods for the determination of the OA background concentrations in plasma have been summarized (reviewed in Petrarulo et al. 1998; further citations in Hönow et al. 2002). The reported OA plasma concentrations in healthy subjects range from 0.4 to 6.0 µmol/l (Petrarulo et al. 1998) and from <0.68 to 15.9 µmol/l (Hönow et al. 2002). Petrarulo et al. (1998) recommend either enzymatic determination or HPLC separation followed by conductivity determination. Hönow et al. (2002) used HPLC separation coupled with an enzyme reactor. None of these methods allows distinguishing between isotopically labeled and unlabeled analytes. Therefore, they were not applicable for the present study. The urinary background OA excretion reported here (Table 3) corresponds with the range from 86 to 622 µmol/24 h published by a series of other groups (Wandzilak et al. 1991; Marangella et al. 1992; Holmes et al. 1993; von Unruh et al. 1998; Maeda-Nakai and Ichiyama 2000; Keßler

et al. 2002; Siener and Hesse 2002). Possible sources for background OA include GA and endogenous glyoxylic acid (Poore et al. 1997), catabolism of ascorbic acid (Levine et al. 1996) and uptake via food (Hönow and Hesse 2002). The contribution of intermediary metabolism to background urinary OA has been estimated to be about 50% (Williams and Wandzilak 1989).

Kinetics

Derivation of kinetic parameters: The concentration-time courses of $^{13}C_2$-EG and $^{13}C_2$-GA in plasma of volunteers A and B can be used to predict some important kinetic parameters, using a one-compartment model (for details see e.g. Filser 1996). For a constant uptake rate, half the plateau concentration is reached after the first half-life (A: 2.1 h; (B) 2.6 h). Consequently, from Fig. 3 it becomes evident that the $^{13}C_2$-EG plateau concentrations in blood amount to 14.4 µmol/l (A) and 24.0 µmol/l (B) considering the "constant" inhalation rates of 358 µmol/h (A) and 335 µmol/h (B). The distribution volume (V_d) – related to the concentration in plasma - can be obtained using the plateau concentration (PC) and the half-life ($T_{1/2}$) since it is given by the expression: V_d = inhalation rate x $T_{1/2}$ / (ln2 x PC). The distribution volumes are obtained to be 75 l (A) and 52 l (B), or 0.78 l/kg bw (A) and 0.91 l/kg bw (B). These values match with those of 0.5 – 0.8 l/kg bw (reviewed in Eder et al. 1998) and are in agreement with the assumption that the highly water soluble EG (Merck 1996) distributes predominantly into the aqueous phase of the body. Longer half-lives between 3.0 and 8.6 h have been derived from cases of EG intoxication where EG concentrations in blood were in the millimolar range (reviewed in Eder et al. 1998).

These differences likely result from saturation of EG metabolism occurring at such high concentrations.

Concerning $^{13}C_2$-GA, such kinetic calculations cannot be made, since the production rate and the distribution volume of this second $^{13}C_2$-EG metabolite are not available from the measured data. However, a worst-case estimate for the maximum $^{13}C_2$-GA concentration reachable as a consequence of an 8-h exposure to $^{13}C_2$-EG can be done from the linearly increasing parts of the measured $^{13}C_2$-GA concentration and assuming a further continuous linear increase of the $^{13}C_2$-GA concentration with the $^{13}C_2$-EG-exposure time. In the present experiments, the 4-h $^{13}C_2$-EG exposures of volunteers A and B led to maximum $^{13}C_2$-GA plasma concentrations of 0.9 µmol/l and 1.8 µmol/l, respectively (Figure 4). For 8-h $^{13}C_2$-EG exposures and considering the above given $^{13}C_2$-EG inhalation rates, a linear increase of the maximum $^{13}C_2$-GA concentrations would lead to 1.8 (A) and 3.6 (B) µmol/l plasma. It has to be stressed that these values are overestimates, since the increase of the unknown $^{13}C_2$-GA production rate is not constant but becomes smaller with the rising $^{13}C_2$-EG concentration. From the observation that in both volunteers the half-lives of $^{13}C_2$-GA were very similar (A) and identical (B) to those of the metabolic precursor $^{13}C_2$-EG, one can conclude that the elimination rate of $^{13}C_2$-GA is determined by its production from $^{13}C_2$-EG. Consequently, even a shorter half-life of GA should be expected if GA would be administered as such.

Kinetic extrapolations: Based on the above data, an extrapolation to workplace conditions according to MAK (10 ppm, 8 h, alveolar ventilation 20 l/min/70 kg bw, Åstrand 1983) can be carried out. Allometrically, the alveolar ventilation is obtained for A to be 24.7 l/min (20 x (bw of A/70)$^{2/3}$) and for B to be 17.4 l/min (20 x (bw of

B/70)$^{2/3}$). Using these values together with the individual volumes of distribution and the obtained half-lives, an exposure to 10 ppm EG (25.7 mg/m^3 = 0.414 µmol/l) should result in maximum EG plasma concentrations of 23.0 µmol/l (A) and 27.5 µmol/l (B) reached at 8 h of exposure. Taking into account that the EG inhalation rate of volunteer A (24.7 x 60 x 0.414 = 614 µmol/h) would be 1.72 times that of the present 4-h exposure (358 µmol/h), the resulting maximum (worst-case) GA plasma concentration would amount to 3.1 µmol/l (1.72 x 1.8), which has to be added to the GA background concentration. Corresponding calculations yield for volunteer B a maximum increase over the background GA plasma concentration of 4.6 µmol GA/l. These values represent 12.0% (A) and 16.3% (B) of the corresponding background GA plasma concentrations.

Based on the relative urinary excretion data of $^{13}C_2$-GA and $^{13}C_2$-OA given in Table 3, corresponding increases over the urinary backgrounds resulting from EG uptake of 4.91 mmol (A) and 3.46 mmol (B) - equivalent to the 8-h exposure to 10 ppm - are predicted to be for GA and OA 12.7% and 1.7% (A) and 36.7% and 5.4% (B), respectively.

Conclusion

Workplace exposure to EG vapors at MAK conditions (10 ppm, 8 h/d, 50 W) leads in a reference man of 70 kg bw to a daily EG uptake of 247 mg. The resulting additional maximum GA and OA burdens are in the range of or below the unavoidable background levels. Furthermore, maximum plasma concentrations of EG and GA are rapidly decreasing after end of exposure, which becomes obvious from their short

half-lives. Hereof, it has to be derived also that both compounds cannot accumulate in daily exposed humans (MAK conditions). According to our data, an 8-h exposure to 10 ppm EG could lead to a maximum increase of the GA plasma concentration of less than 5 µmol/l. With the highest background GA concentration of 32.9 µmol/l, reported Chalmers et al. (1984), this would summarize to about 38 µmol/l. Considering that in rats the lowest NOEL for EG-induced nephrotoxicity was 80 mg/kg bw/d (Blood 1965), which leads to maximum GA concentrations of 144 µmol/l blood and that the NOEL for developmental toxicity was 500 mg/kg bw resulting in a maximum GA concentration in blood of 1723 µmol/l, we conclude that GA and OA induced nephrotoxic or developmental toxic effects resulting from human exposure to 10 ppm EG are highly unlikely to occur.

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Tables

Table 1: Doses of $^{13}C_2$-EG inhaled

Volunteer	Total dose [mmol]	Normalized dose [mg/kg bw]
A	1.43	0.96
B	1.34	1.51

Table 2: $^{13}C_2$-Labeled urinary EG, GA and OA expressed as a percentage of inhaled dose $^{13}C_2$-EG

Volunteer	Amount in urine as a percentage of the inhaled dose of $^{13}C_2$-EG		
	$^{13}C_2$-EG	$^{13}C_2$-GA	$^{13}C_2$-OA
A	6.4%	0.70%	0.08%
B	9.3%	0.92%	0.28%

Table 3: Unlabeled acids excreted in urine over 24 h and urinary excretion of $^{13}C_2$-labeled GA and OA after inhalation of about 1.4 mmol $^{13}C_2$-EG given as a percentage of the corresponding unlabeled acids

Volunteer	Background acid in urine		$^{13}C_2$-Labeled acid	
	GA [µmol/24 h]	OA [µmol/24 h]	$^{13}C_2$-GA	$^{13}C_2$-OA
A	274	215	3.7%	0.5%
B	88	177	14.2%	2.1%

Legends

Figure 1: Metabolism of ethylene glycol

Figure 2: Schematic set-up for inhalation exposure to $^{13}C_2$-EG vapor

Figure 3: $^{13}C_2$-EG in plasma during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor

Figure 4: $^{13}C_2$-GA in plasma during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor

Figure 5: Background EG and $^{13}C_2$-EG in urine during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor, cumulative excretion, means ± S.D., n = 3

Figure 6: Background GA and $^{13}C_2$-GA in urine during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor, cumulative excretion, means ± S.D., n = 3

Figure 7: Background OA and $^{13}C_2$-OA in urine during and after a 4-h inhalation exposure to $^{13}C_2$-EG vapor, cumulative excretion, means ± S.D., n = 3

Figure 1

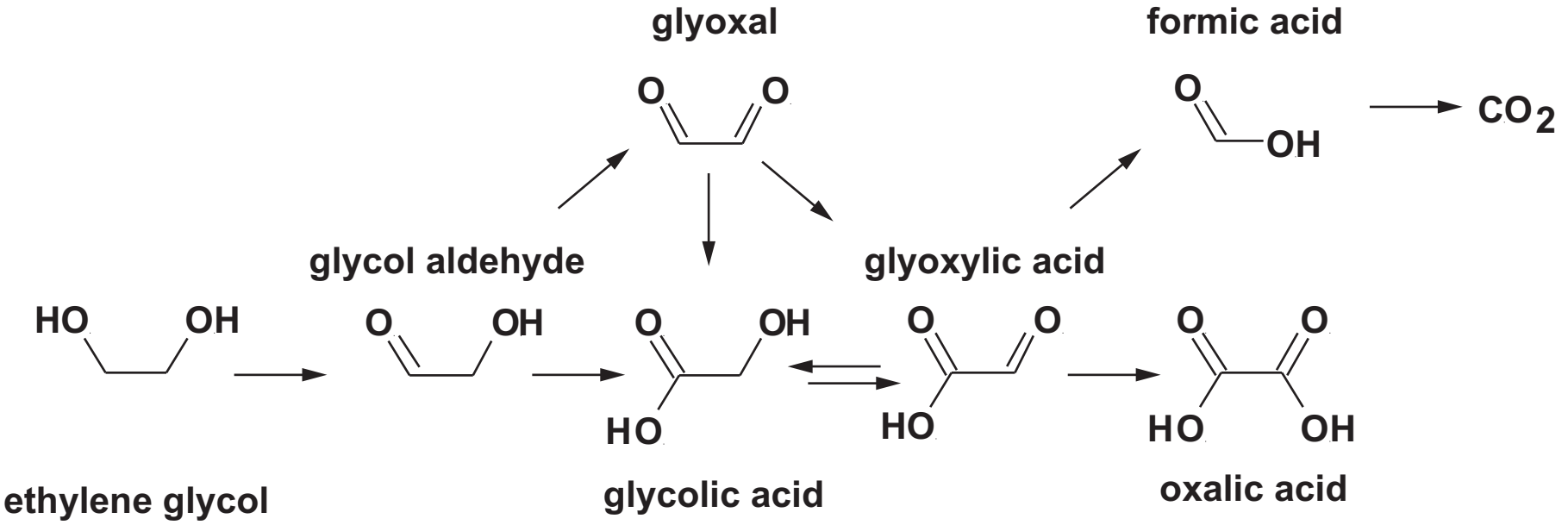

glyoxal
formic acid
CO2
glycol aldehyde
glyoxylic acid
HO
OH
ethylene glycol
glycolic acid
oxalic acid

Figure 2

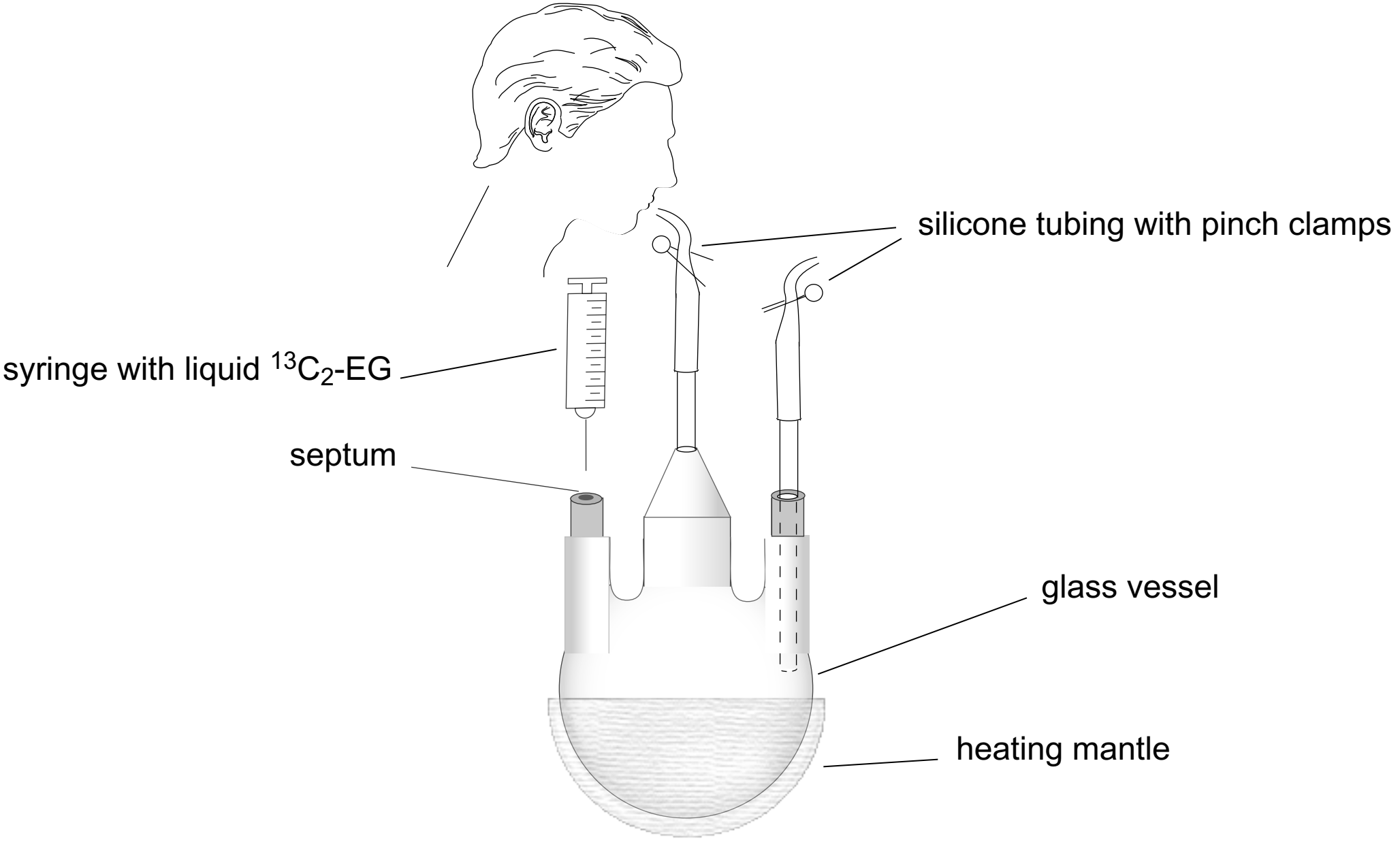

silicone tubing with pinch clamps
syringe with liquid $^{13}C_2$-EG
septum
glass vessel
heating mantle

Figure 3

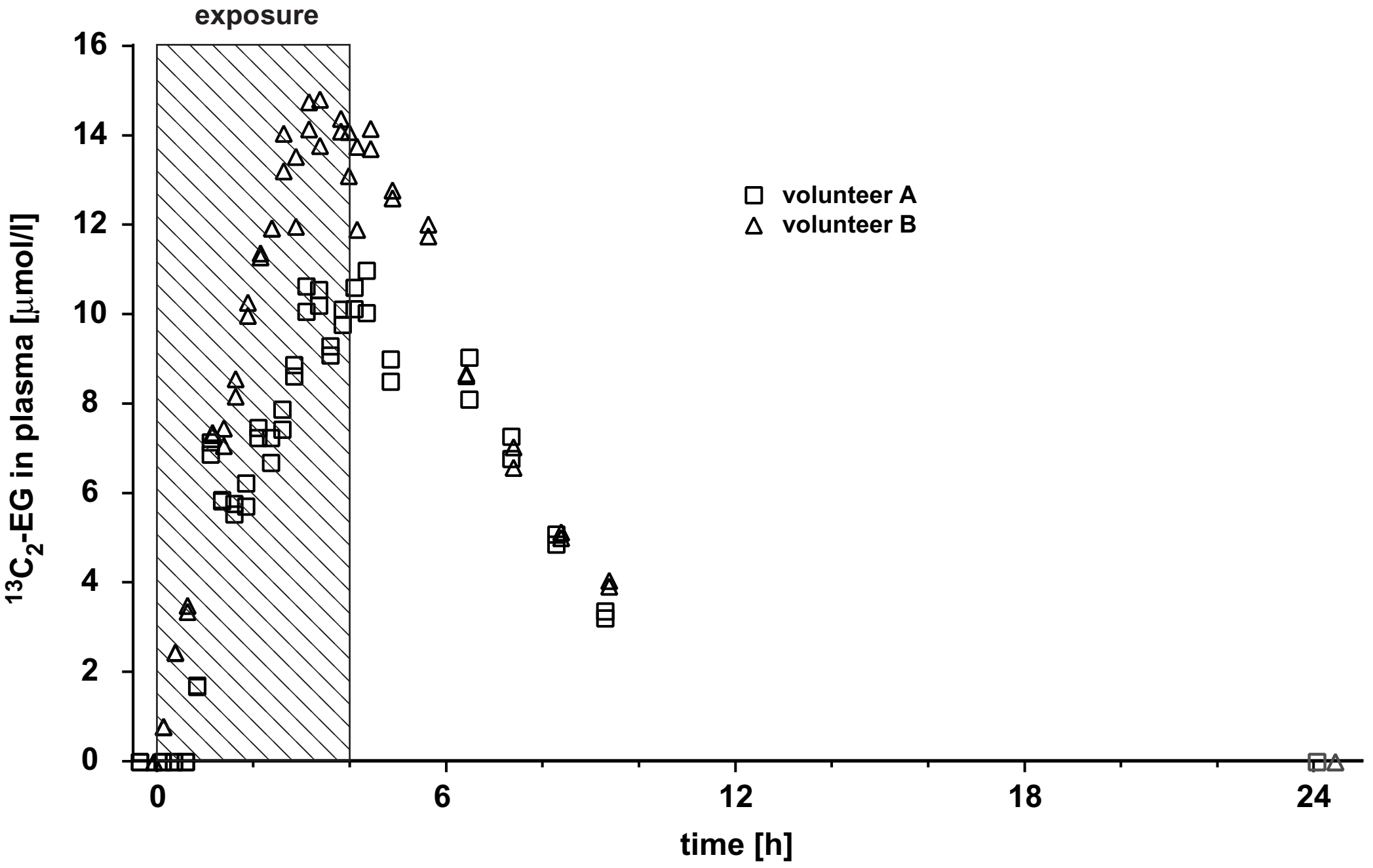

exposure
16
14
12
10
8
6
4
2
0
$^{13}C_2$-EG in plasma [µmol/l]
volunteer A
volunteer B
0
6
12
18
24
time [h]

Figure 4

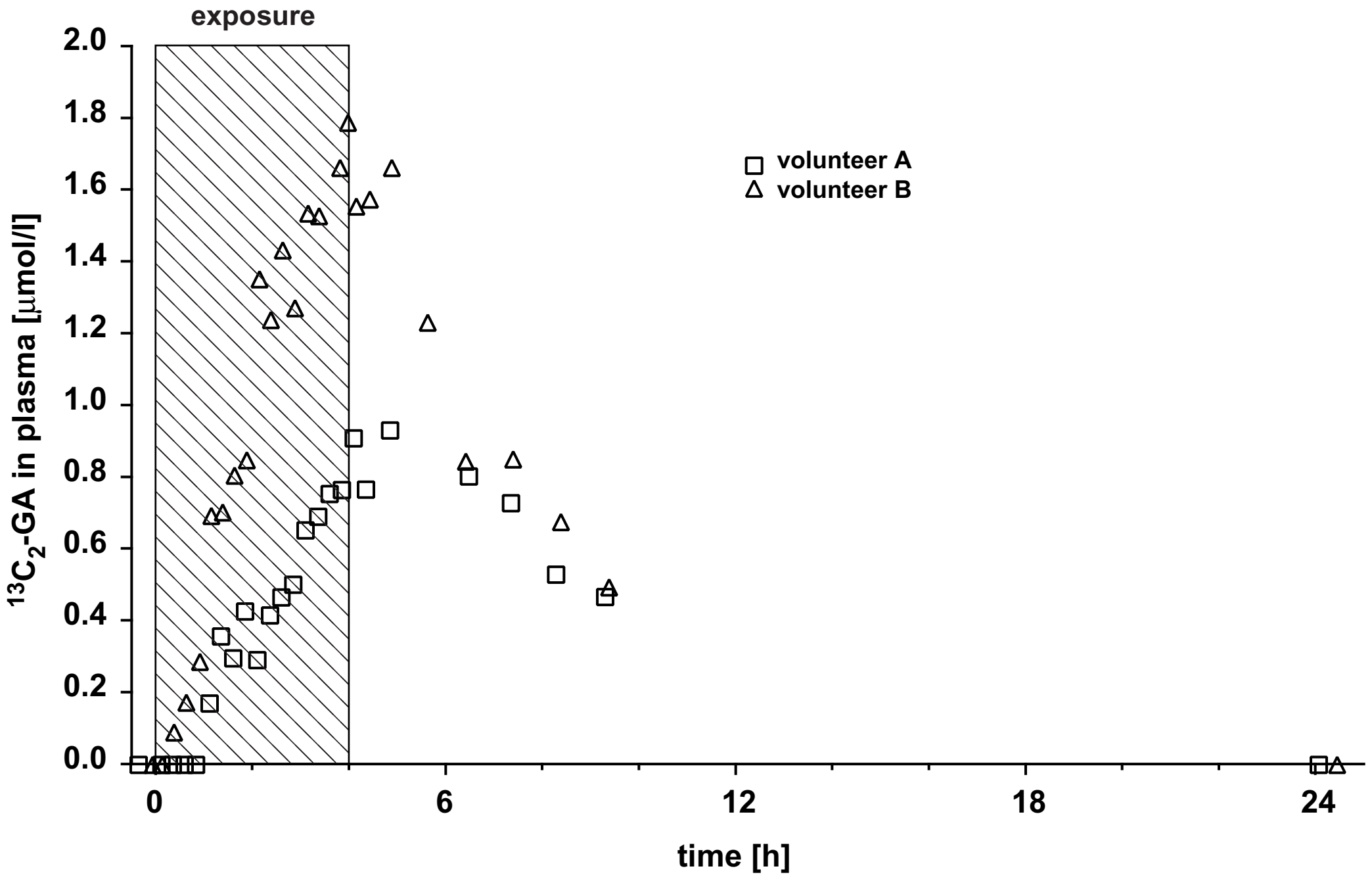

exposure
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
$^{13}C_2$-GA in plasma [µmol/l]
volunteer A
volunteer B
0
6
12
18
24
time [h]

Figure 5

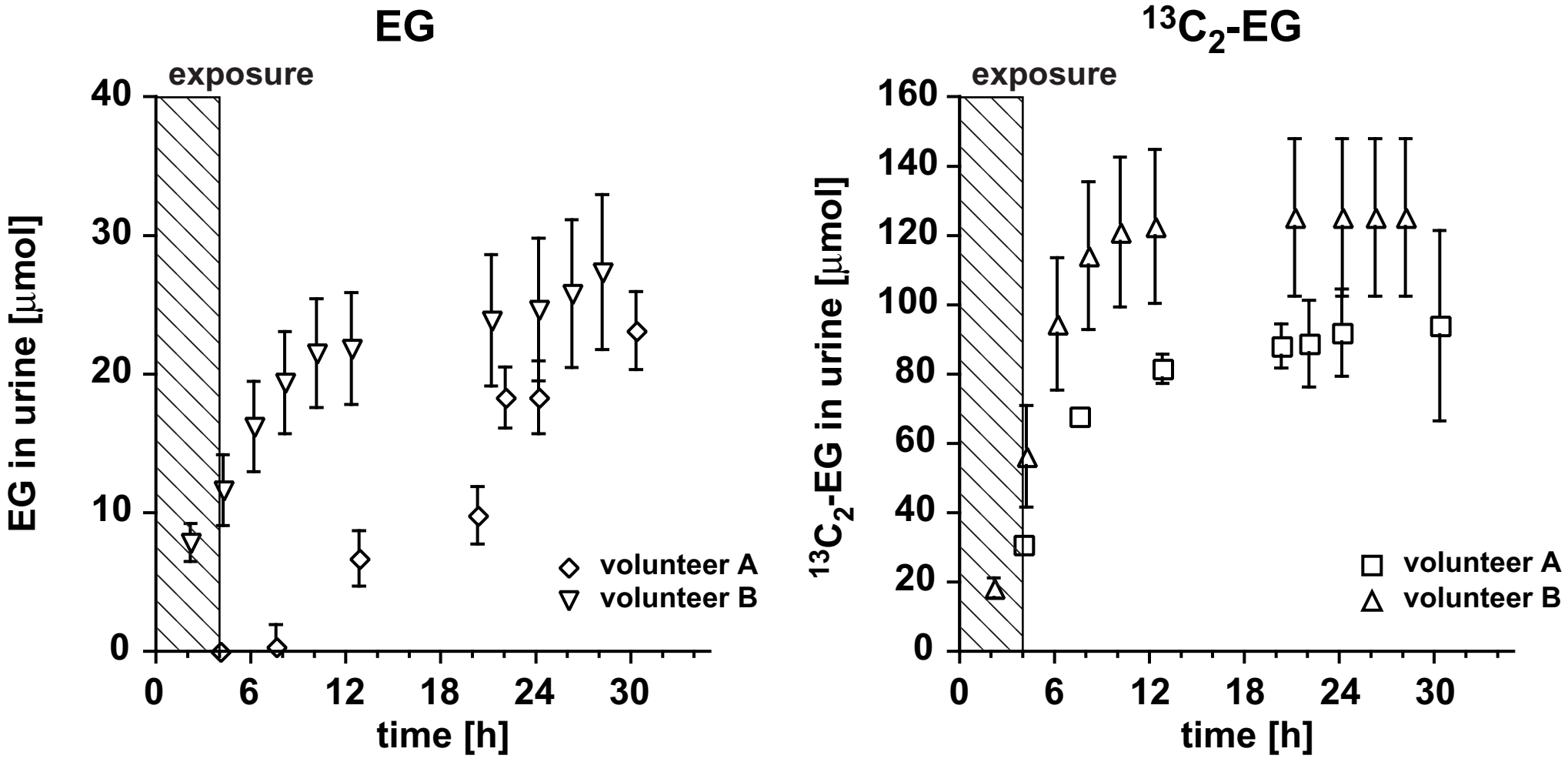

EG
exposure
EG in urine [µmol]
40
30
20
10
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B
$^{13}C_2$-EG
exposure
$^{13}C_2$-EG in urine [µmol]
160
140
120
100
80
60
40
20
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B

Figure 6

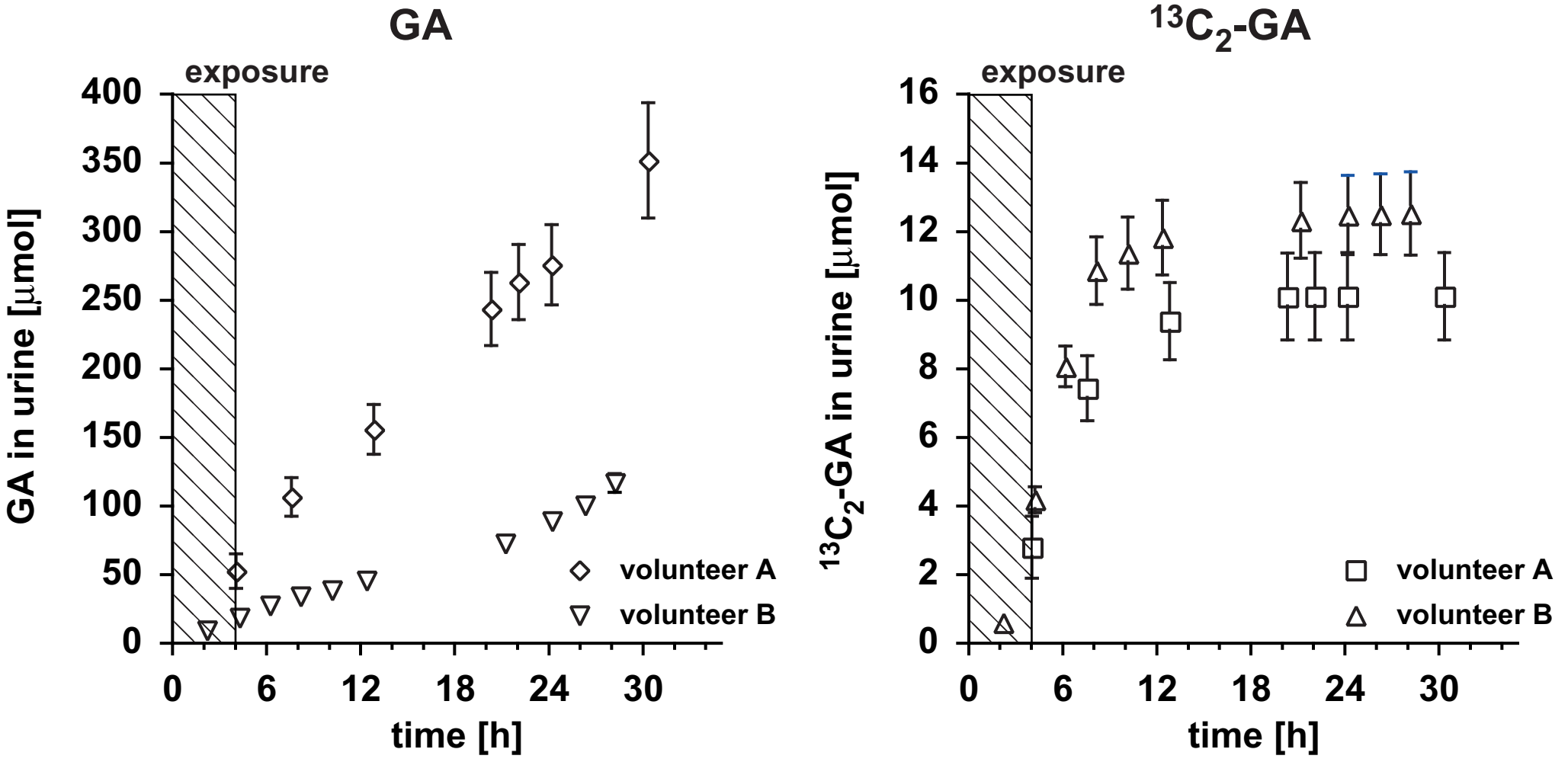
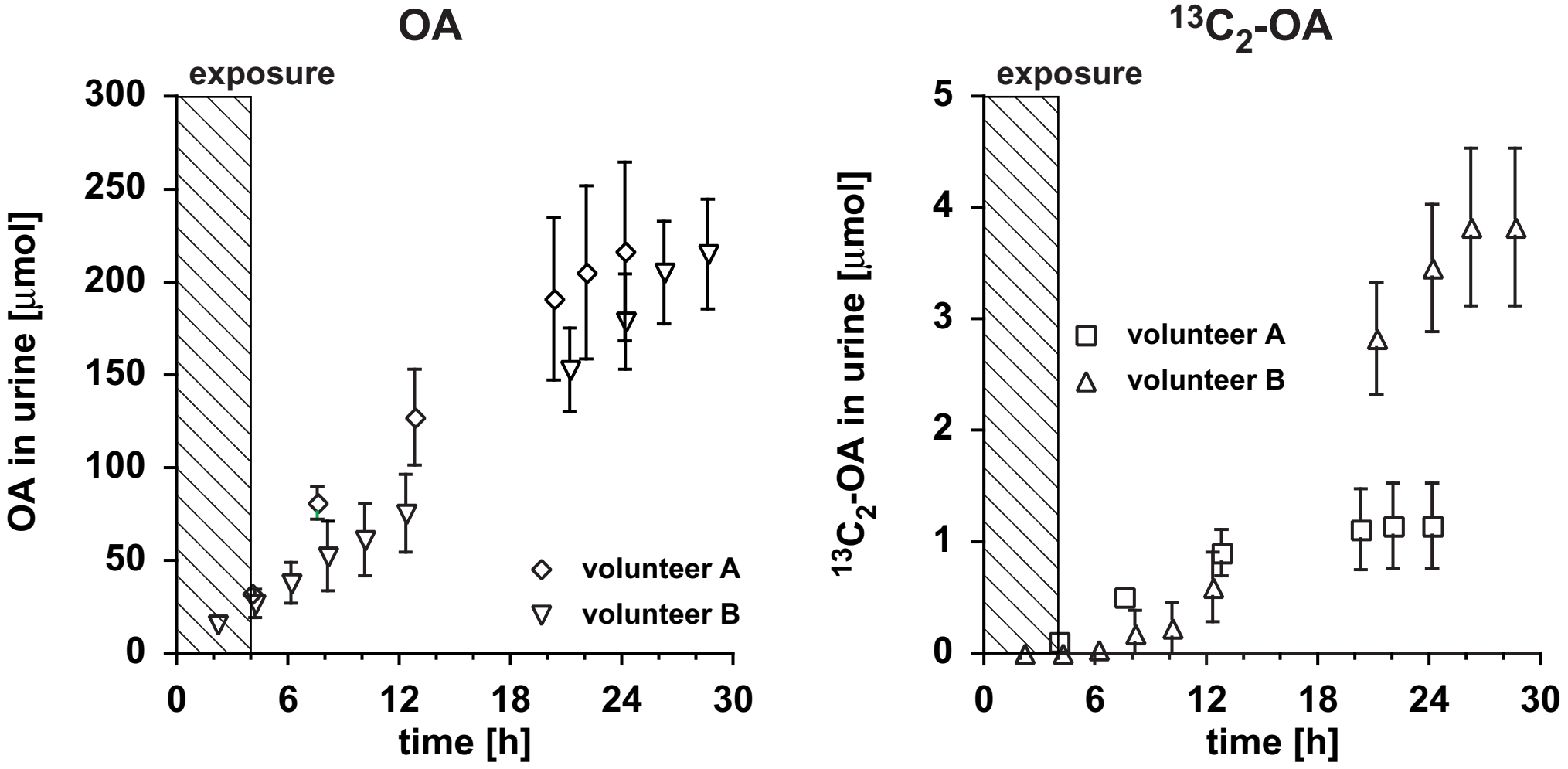

GA
exposure
GA in urine [µmol]
400
350
300
250
200
150
100
50
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B
$^{13}C_2$-GA
exposure
$^{13}C_2$-GA in urine [µmol]
16
14
12
10
8
6
4
2
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B

Figure 7


OA
exposure
OA in urine [μmol]
300
250
200
150
100
50
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B
$^{13}C_2$-OA
exposure
$^{13}C_2$-OA in urine [μmol]
5
4
3
2
1
0
0
6
12
18
24
30
time [h]
volunteer A
volunteer B